

What is claimed is:

1. A vitamin K-dependent polypeptide comprising a modified GLA domain that enhances membrane binding affinity and activity of said polypeptide relative to a corresponding native vitamin K-dependent polypeptide, said modified GLA domain comprising at least one amino acid substitution, wherein said polypeptide is one that inhibits clot formation. *result*

2. The polypeptide of claim 1, wherein said GLA domain is from amino acid 1 to about amino acid 45.

3. The polypeptide of claim 1, wherein said at least one amino acid substitution is at amino acids 2, 5, 9, 11, 12, 29, 33, 34, 35, or 36.

4. The polypeptide of claim 1, wherein said at least one amino acid substitution is at amino acids 2, 5, or 9.

5. The polypeptide of claim 1, wherein said at least one amino acid substitution is at amino acids 11 or 12.

6. The polypeptide of claim 1, wherein said at least one amino acid substitution is at amino acids 29 or 33.

7. The polypeptide of claim 1, wherein said at least one amino acid substitution is at amino acids 34, 35, or 36.

8. The polypeptide of claim 1, wherein said polypeptide comprises Protein C or Activated Protein C.

9. The polypeptide of claim 8, wherein said at least one amino acid substitution comprises a glycine residue at amino acid 12.

10. The polypeptide of claim 9, wherein said at least one amino acid substitution further comprises a glutamic acid residue at amino acid 33 and an aspartic acid or glutamic acid residue at amino acid 34.

11. The polypeptide of claim 9, wherein said at least one amino acid substitution further comprises an aspartic acid or glutamic acid residue at amino acid 35.

12. The polypeptide of claim 11, wherein said at least one amino acid substitution further comprises or a glutamic acid residue at amino acid 36.

13. The polypeptide of claim 9, wherein said at least one amino acid substitution further comprises a glutamine or a glutamic acid residue at amino acid 11.

14. The polypeptide of claim 9, wherein said at least one amino acid substitution further comprises a phenylalanine residue at amino acid 29.

15. The polypeptide of claim 1, wherein said polypeptide comprises active-site modified Factor VIIa.

16. The polypeptide of claim 15, wherein said at least one amino acid substitution comprises a glutamine residue at amino acid 11 and a glutamic acid residue at amino acid 33.

17. The polypeptide of claim 15, wherein said at least one amino acid substitution further comprises an aspartic acid or a glutamic acid residue at amino acid 35.

18. The polypeptide of claim 1, wherein said polypeptide is Protein S.

19. The polypeptide of claim 18, wherein said at least one amino acid substitution comprises an isoleucine, leucine, valine, or phenylalanine residue at amino acid 9.

20. The polypeptide of claim 19, wherein said at least one amino acid substitution further comprises an aspartic acid or glutamic acid residue at amino acids 34 or 35.

21. The polypeptide of claim 18, wherein said at least one amino acid substitution comprises a phenylalanine residue at amino acid 5.

22. The polypeptide of claim 18, wherein said polypeptide further comprises an amino acid substitution in the thrombin-sensitive loop.

23. The polypeptide of claim 22, wherein said amino acid substitution in the thrombin sensitive loop is at amino acid 49, 60, or 70.

24. The polypeptide of claim 1, wherein said polypeptide is active-site modified Factor IXa.

25. The polypeptide of claim 24, wherein said at least one amino acid substitution comprises a phenylalanine at amino acid 29 or amino acid 34.

26. The polypeptide of claim 24, wherein said at least one amino acid substitution comprises a phenylalanine, leucine, or isoleucine residue at amino acid 5.

27. The polypeptide of claim 24, wherein said at least one amino acid substitution comprises an aspartic acid or glutamic acid residue at amino acids 34 or 35.

28. The polypeptide of claim 1, wherein said vitamin K-dependent polypeptide further comprises an inactivated cleavage site.

29. The polypeptide of claim 28, wherein said polypeptide comprises factor VII.

30. The polypeptide of claim 29, wherein said inactivated cleavage site comprises a substitution of an alanine residue at amino acid 152.

31. The polypeptide of claim 1, wherein said polypeptide is active site modified factor Xa.

32. The polypeptide of claim 31, wherein said at least one amino acid substitution comprises a glutamine at amino acid 11.

33. The polypeptide of claim 31, wherein said at least one amino acid substitution comprises an aspartic acid or glutamic acid residue at amino acid 35.

34. The polypeptide of claim 1, wherein said polypeptide is protein Z.

35. The polypeptide of claim 34, wherein said at least one amino acid substitution comprises an asparagine or glutamine residue at amino acid 2.

36. The polypeptide of claim 35, wherein said at least one amino acid substitution comprises an aspartic acid or glutamic acid residue at amino acids 34, 35, or 36.

37. A vitamin K-dependent polypeptide comprising a modified GLA domain that enhances membrane binding affinity and activity of said polypeptide relative to a corresponding native vitamin K-dependent polypeptide, said modified GLA domain comprising at least one amino acid insertion at amino acid 4.

38. The polypeptide of claim 37, wherein said polypeptide is selected from the group consisting of factor VII or factor VIIa, protein C or activated protein C, factor X or factor Xa, and protein S.

39. The polypeptide of claim 37, wherein said polypeptide is factor VII or factor VIIa.

40. The polypeptide of claim 39, wherein said amino acid insertion comprises a tyrosine or glycine residue.

41. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an effective amount of a vitamin K-dependent polypeptide, wherein said vitamin K-dependent polypeptide comprises a modified GLA domain that enhances membrane

binding affinity and activity of said polypeptide relative to a corresponding native vitamin K-dependent polypeptide, said modified GLA domain comprising at least one amino acid substitution, and wherein said vitamin K-dependent polypeptide inhibits clot formation.

42. The pharmaceutical composition of claim 41, wherein said polypeptide is Protein C or Activated Protein C.

43. The pharmaceutical composition of claim 41, wherein said at least one amino acid substitution comprises a glycine at amino acid 12.

44. The pharmaceutical composition of claim 43, wherein said at least one amino acid substitution further comprises a glutamic acid residue at amino acid 33 and an aspartic acid residue at amino acid 34.

45. The pharmaceutical composition of claim 43, wherein said at least one amino acid substitution further comprises an aspartic acid or glutamic acid residue at amino acids 35 or 36.

46. The pharmaceutical composition of claim 41, wherein said polypeptide is active-site modified Factor VIIa.

47. The pharmaceutical composition of claim 46, wherein said at least one amino acid substitution comprises a glutamine residue at amino acid 11 and a glutamic acid residue at amino acid 33.

48. The pharmaceutical composition of claim 41, wherein said polypeptide is Protein S.

49. The pharmaceutical composition of claim 41, wherein said polypeptide is active-site modified Factor IXa.

50. The pharmaceutical composition of claim 41, wherein said composition further comprises an anticoagulant agent.

51. A mammalian host cell comprising a vitamin K-dependent polypeptide, said vitamin K-dependent polypeptide comprising a modified GLA domain that enhances membrane binding affinity and activity of said polypeptide relative to a corresponding native vitamin K-dependent polypeptide, said modified GLA domain comprising at least one amino acid substitution, wherein said polypeptide is one that inhibits clot formation.

52. A method of decreasing clot formation in a mammal comprising administering an amount of a vitamin K-dependent polypeptide effective to decrease clot formation in said mammal, wherein said vitamin K-dependent polypeptide comprises a modified GLA domain that enhances membrane binding affinity and activity of said polypeptide relative to a corresponding native vitamin K-dependent polypeptide, said modified GLA domain comprising at least one amino acid substitution.

53. The method of claim 52, wherein said polypeptide is Protein C or Activated protein C.

54. The method of claim 52, wherein said polypeptide is active-site modified Factor VIIa.

55. The method of claim 52, wherein said polypeptide is active-site modified Factor IXa.

56. The method of claim 52, wherein said polypeptide is Protein S.

57. A method for identifying a vitamin K-dependent polypeptide having enhanced membrane binding affinity and activity comprising:

(a) modifying the GLA domain of said vitamin K-dependent polypeptide, wherein said modifying comprises substituting at least one amino acid in said GLA domain;

- b) monitoring membrane binding affinity and activity of said vitamin K-dependent polypeptide having said modified GLA domain; and
- (c) identifying said modified vitamin K-dependent polypeptide as having enhanced membrane binding affinity and activity if membrane binding affinity and activity of said modified vitamin K-dependent polypeptide is enhanced relative to a corresponding native vitamin K-dependent polypeptide.

58. The method of claim 57, wherein said at least one amino acid substitution is at amino acids 2, 5, 9, 11, 12, 29, 33, 34, 35, or 36.

59. The method of claim 57, wherein said modified vitamin K-dependent polypeptide increases clot formation.

60. The method of claim 57, wherein said modified vitamin K-dependent polypeptide inhibits clot formation.

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